ing new angle to transposon regulation. The relatively low TnsE binding affinity for the β clamp suggests that it has evolved to use the replication factor but not to appropriate it entirely at the expense of other β clamp binding partners and functions. In the wake of the new findings of Parks et al., it is now important to determine how such interactions between transposon proteins and the replication machinery occur, how they are temporally integrated into DNA replication and other processes coordinated by the β clamp, and whether other host proteins are also required.

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Breast Cancer Stem Cells: Eradication by Differentiation Therapy?

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During metastasis, migrating breast cancer stem cells undergo a loss of polarity leading to an epithelial-to-mesenchymal transition (EMT). Gupta et al. (2009) use this attribute of cancer stem cells to develop a high-throughput screen, which successfully identifies small molecules that specifically inhibit cancer stem cell proliferation through the induction of differentiation.

Although tumor metastases are the cause of death in more than 80% of human cancer patients, the molecular mechanisms underpinning metastasis are still poorly understood. However, one theme that has emerged from recent work is that metastasis involves defects in the molecular machines responsible for epithelial polarity and hence for the epithelial-to-mesenchymal transition (EMT) (Kalluri and Weinberg, 2009). Epithelial cell polarity is established by multiple cellular processes, including polarized trafficking of cytoskeletal and plasma membrane proteins, the maintenance of a diffusion barrier (tight junctions) (Humbert et al., 2008), and a 3D organization machinery that integrates extracellular information from receptors, adhesion proteins, and neighboring cells (Tanos and Rodriguez-Boulan, 2008). Hence, the loss of epithelial polarity during EMT can result from altered regulation of many different signaling pathways, transcription factors, chromatin regulators, and proteins involved in cell adhesion and polarity (Kalluri and Weinberg, 2009; Tanos and Rodriguez-Boulan, 2008). Indeed, the list of oncogenes and tumor suppressors that modulate cell polarity is long and growing (Humbert et al., 2008; Tanos and Rodriguez-Boulan, 2008). In recent work, it has been shown that epithelial stem cells may undergo EMT and that EMT induction endows epithelial cells with salient features of stem cells. These cells can also exhibit properties of cancer stem cells upon overexpression of oncogenic Ras (Mani et al., 2008). In their current work, published in this issue of *Cell*, Gupta et al. (2009) use these newly discovered attributes of mammary epithelial cells (that is, induction of EMT and stem cell features by defined genetic alterations) to establish a high-throughput screen for compounds that selectively target cancer stem cells.

Gupta et al. use telomerase-immortalized human mammary epithelial (HMLE) cells, in which knockdown of E-cadherin by RNA interference promotes EMT and the acquisition of features typical of cancer stem cells, including high levels of CD44, low levels of CD24, and the capacity to form mammospheres (Figure 1). Cells depleted of E-cadherin also show increased resistance to several established tumor chemotherapeutics. In this respect, they resemble human breast carcinoma stem cells that contribute to tumor relapse in vivo. Gupta et al. establish that these cells are ideally suited for a high-throughput, cellular screen for compounds that selectively eliminate cancer stem cells (Figure 1). The most extensively

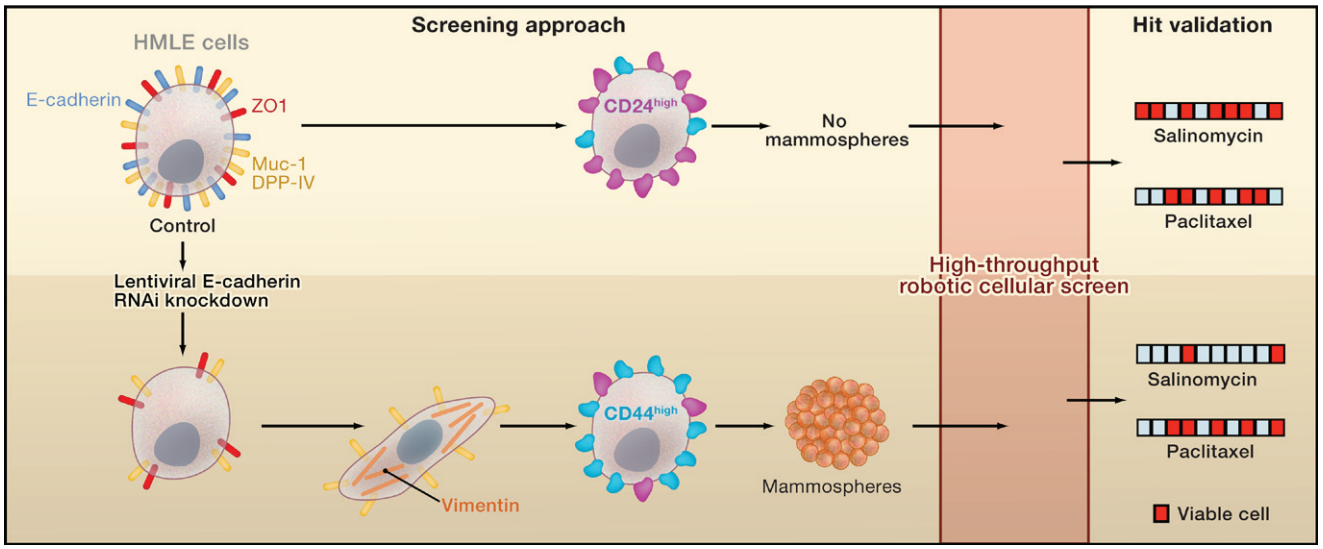

Figure 1. Salinomycin Targets Breast Cancer Stem Cells

Depicted is the screening approach used by Gupta et al. (2009). Control human mammary epithelial (HMLE) cells express the basolateral marker E-cadherin and high levels of CD24. These cells do not form mammospheres in suspension culture. In contrast, HMLE cells treated with a short hairpin RNA targeting E-cadherin undergo changes consistent with an epithelial to mesenchymal transition (EMT), including upregulation of the mesenchymal marker vimentin. These cells exhibit features of cancer stem cells, including high levels of CD44 expression and the ability to form mammospheres in suspension culture. These two cell types were then subjected to a high-throughput chemical screen measuring cell viability, which led to the identification of salinomycin as a compound that targets breast cancer stem cells selectively.

validated hit from their screen is salinomycin, a highly selective potassium ionophore, facilitating bidirectional ion flux (Mitani et al., 1975). Salinomycin selectively impairs the viability of cells with features of cancer stem cells. In artificial mixtures of Ras-transformed HMLE cells in which some cells exhibit EMT and cancer stem cell features and some cells do not, salinomycin selectively eradicates cells with cancer stem cell properties. In contrast, established cancer chemotherapeutics (such as paclitaxel) have the opposite effect, even leading to cancer stem cell enrichment. In addition, in an orthotopic mouse model of lung metastasis using 4T1 murine carcinoma cells, salinomycin fully reverses the partial EMT-phenotype of these cells in that they adopt an epithelial phenotype. Although the mechanism of action for salinomycin is not yet clear, it appears that it might induce terminal epithelial differentiation accompanied by cell-cycle arrest rather than trigger cytotoxicity. This is consistent with evidence showing that salinomycin does not block proliferation in several other human mammary carcinoma cell lines.

Importantly, salinomycin also eradicates cells with cancer stem cell properties in mice. Tumors derived from 4T1 cells (or from Ras-transformed HMLE cells) form less efficiently in mice after treatment of the cells with salinomycin. Interestingly, in mice inoculated with SUM159 human breast cancer cells, treatment with salinomycin or paclitaxel delays tumor formation by 14 days. Furthermore, salinomycin induces expression of plasma membrane-E-cadherin in these tumors, providing further evidence that salinomycin might eliminate cancer stem cells by inducing their differentiation. Significantly, salinomycin also suppresses the metastasis of 4T1 cells to the lung. This differs from paclitaxel, which increases the prevalence of lung metastases in this model. Thus, salinomycin might selectively suppress metastasis by inducing differentiation in the migrating cancer stem cells that have undergone EMT.

The authors employ then global expression profiling to show that the fraction of Ras-transformed HMLE cells with properties of cancer stem cells have a gene expression signature found in natural mammary stem cells and in cancer stem cells from human tumors. They compared three cell states: (1) paclitaxel treatment versus salinomycin treatment of Ras-transformed HMLE cells, (2) mammospheres versus differentiation cultures of primary human mammary epithelial cells, and (3) cells expressing high levels of CD44 versus those expressing high levels of CD24 as sorted from normal human mammary glands and mammary carcinomas. They report 25 genes that are upregulated and 14 that are downregulated consistently in all of the above comparisons. This analysis clearly establishes that the Ras-transformed HMLE cells eliminated by salinomycin have a gene expression signature characteristic of mammary and cancer stem cells.

Established breast cancer therapies often fail to achieve long-term patient survival, possibly because of tumor relapse as a result of chemotherapy-resistant mammary cancer stem cells. Thus, new therapeutic strategies to specifically target these cancer stem cells are urgently required. Gupta et al. (2009) significantly advance this field by presenting the first clear proof of principle that it is feasible to screen for drugs that specifically target breast cancer stem cells. The approach they chose evolved from the concept that normal mammary gland stem cells and cancer stem cells display a highly plastic epithelial phenotype, which allows them to undergo EMT in cell culture (Mani et al., 2008). It remains unclear, however, by which mechanisms salinomycin selectively targets cells after EMT. Salinomycin is

used as an antibiotic against eukaryotic parasites in animals and inhibits cartilage degradation during bone development (Peters et al., 2002). Given that it is a highly selective potassium ionophore, salinomycin may interfere with the function of potassium channels in cancer stem cells. It has been shown that tumor cells express elevated levels of various types of K^+ channels. Their overexpression enhances proliferation, and drugs acting as channel blockers inhibit cell proliferation (Le Guennec et al., 2007; Zhang et al., 2009). Perhaps more importantly, certain K^+ channels regulate migration. In a similar vein, certain G protein-coupled K^+ channels are overexpressed in breast cancer lymph node metastases (Zhang et al., 2009). Given the importance of the cell polarity machinery to cell migration (Etienne-Manneville, 2008), it is tempting to speculate that K^+ channels targeted by salinomycin have a critical function in epithelial polarity and metastasis, which can be deregulated by salinomycin.

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Plant Phase Transitions Make a SPLash

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During post-embryonic development, plants undergo a series of phase transitions, from juvenile to adult and from the vegetative to the reproductive phase. Recent findings reported in *Cell* (Wang et al., 2009; Wu et al., 2009) and *Developmental Cell* (Yamaguchi et al., 2009) reveal how microRNAs and their transcription factor targets coordinate these phase transitions.

In plants, the juvenile and adult phases of vegetative development can be distinguished by leaf morphology, and the reproductive phase of development can be distinguished by the production of flowers. SQUAMOSA PROMOTER BINDING-LIKE (SPL) transcription factors are regulated by the microRNA *miR-156* and influence the transitions between these developmental phases (Schwab et al., 2005; Wu and Poethig, 2006). Intricate models have been proposed to explain the regulation of the developmental transitions between these phases, particularly between the adult vegetative and reproductive phases (Baurle and Dean, 2006). However, SPL transcription factors are often excluded from these models because their extreme genetic redundancy and the complexity of their regulation by microRNAs have made it difficult to pinpoint their exact functions in development. Wang et al. (2009) and Wu et al. (2009), reporting in this issue of *Cell*, and Yamaguchi et al. (2009), reporting in a recent issue of *Developmental Cell*, now use a variety of elegant approaches to demonstrate precise functions for SPL transcription factors and their regulation by microRNAs at different stages of plant development.

The involvement of SPL transcription factors in the vegetative phase transition first became clear by studying *miR-156*, a microRNA whose overexpression prolongs the juvenile phase in both maize and the model plant *Arabidopsis thaliana* (Wu and Poethig, 2006; Chuck et al., 2007). The *miR-156* microRNA represses the activity of 10 of the 16 SPL transcription factors in *Arabidopsis*, suggesting that some or all of these proteins promote the transition from the juvenile to the adult phase. In both *Arabidopsis* and maize, *miR-156* expression decreases as the plant ages. Therefore, *miR-156* likely acts during the juvenile phase to repress SPL transcription factors and delay the transition to the adult phase; increasing the abundance of SPL transcription factors shortens the juvenile phase and promotes flowering (Wu and Poethig, 2006). Strikingly, the microRNA *miR-172* shows a complementary pattern of expression, that is, low during the juvenile phase and high during the adult phase. In *Arabidopsis*, *miR-172* represses expression of six members of the APETALA2 (AP2)-like family of transcription factors. Two of these transcription factors—TARGET OF